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EXPERIMENTS ON THE GERMICIDAL ACTION OF COW'S MILK.*

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Since Fokker¹ suggested that fresh cow's milk possessed a germicidal action, the subject has been investigated by many observers and the conclusions arrived at are by no means harmonious. One faction claims that there is no germicidal action, the chief workers being Moro,² Honigman,³ Hesse,⁴ Basenau,⁵ and Stocking.⁶ The opposite opinion has been advocated by Ehrlich and Brieger⁷ who found that antitoxic and bactericidal substances are transferred by means of milk. Among others who have defended the presence of germicidal substances in fresh milk are Park,⁸ Hunziker,⁹ Koning,¹⁰ Kolle,¹¹ and Hippius.¹² The literature has been discussed frequently and thoroughly, so that it seems unnecessary to take up that phase of the subject here.

Experiments made by one of us, the results of which were reported at the meeting of American Bacteriologists in New York in 1906, seemed to show that the germicidal action is at least quite variable, that some bacteria decrease in numbers for several hours; others, however, hold their own, while some even increase slightly from the start. It cannot be denied that there is some restraining action in fresh milk, although the decrease never compares in degree with the germicidal action exerted by blood or blood serum. The results of the work referred to were obtained by inoculating bacteria in suspension into the milk and species were selected chiefly which are known to multiply readily in sterilized milk. It was shown that the objection that many

* Received for publication October 15, 1908.

⁴ *Ztschr. f. Hyg.*, 1894, 17, p. 238.

¹ *Ztschr. f. Hyg.*, 1890, 9, p. 41.

⁵ *Archiv f. Hyg.*, 1895, 23, p. 170.

² *Munch. med. Wchnschr.*, 1891, 48, p. 1770.

⁶ *Storrs Agric. Exper. Station Report*, 1904, p. 89.

³ *Ztschr. f. Hyg.*, 1893, 14, p. 207.

⁷ *Ztschr. f. Hyg.*, 1893, 13, p. 336.

⁸ *N. Y. University Bull. Med. Sc.*, 1901, 1, p. 2.

⁹ *Cornell Univ. Agric. Expr. Station Bull.*, 1901, No. 197.

¹⁰ *Milchwirthsch. Zentralbl.*, 1905, 1, p. 49.

¹¹ *Milch-hygienische Untersuchungen*, Jena, 1904. G. Fischer.

¹² *Jahrbuch f. Kinderheilkunde*, 1905, 11, p. 365.

bacteria which gain access to milk, find milk an unsuitable medium and die, cannot be upheld.

Lately Rosenau and McCoy¹ published experiments which suggest that there is no actual decrease in any case in fresh milk, but that the apparent decrease is due to the presence of agglutinins, which cause the bacteria to clump so as to form a smaller number of colonies as the time progresses. If this be true it is evident that milk serum should agglutinate bacteria in suspension.

The work of Rosenau and McCoy suggested to us a plan of work which was carried out and which it is the purpose of this paper to discuss.

Some preliminary experiments with milk serum made several years ago showed that it actually is capable of agglutinating many species of bacteria, occasionally in dilutions higher than 1:200. The serum was obtained by passing milk through a Berkefeld filter and the filtrate was mixed with suspensions of bacteria in physiological salt solution. The same method was followed in the present experiments and in addition milk was inoculated with suspensions of the identical cultures used for the agglutination tests. The milk was obtained fresh from three cows alternately. The udders were previously washed with a solution of bichloride of mercury and kept covered with a cloth moistened with the same solution during milking. Sterilized wide-mouth glass-stoppered bottles of 250 c.c. capacity served as receptacles. Of this milk 10 c.c. were distributed into each of 10 sterilized culture tubes and the balance of the milk poured on a sterilized Berkefeld filter and the filtrate tested for the presence of agglutinins.

The tubes were treated in the following manner:

One c.c. of each of two tubes was diluted with 99 c.c. of sterilized water and plated. One of these dilutions was shaken moderately, the other vigorously, before plating. Two tubes were then inoculated with a suspension of some organism and a dilution of 1:10,000 prepared from each and plated. One of these tubes was shaken moderately, the other vigorously. Two tubes were then heated for 30 minutes at 56° C., another set of two tubes at 75° C., and a third set at 100°. Each pair after cooling was inoculated with the same organism with which the raw milk had been inoculated and plates prepared from dilutions of 1:10,000, one of each pair shaken moderately, the other vigorously. All tubes were then placed in an incubator at 37° C. and similar

¹ *Jour. Exper. Med.*, 1908, 18, p. 165.




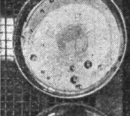






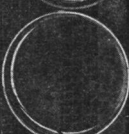






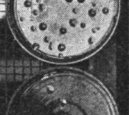


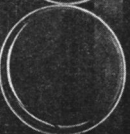


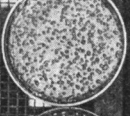

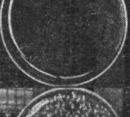


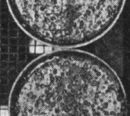


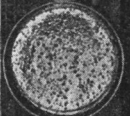
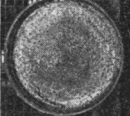
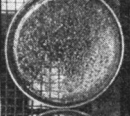






Time of plating after milk- ing	Milk plain- Control	Raw milk inoculated	Milk heated to 56° C. for 30 min. then inocu- lated	Milk heated to 75° C. then inoculated	Milk heated to 100° then inoculated	Raw milk Control	Raw milk inoculated	Milk heated to 56° C. for 30 min. then inoculated	Milk heated to 75° C. then inoculated	Milk heated to 100° C. then inoculated	Time of plating after milk- ing
30 min.											30 min.
3 hours											3 hours
6 hours											6 hours
9 hours											9 hours

FIG. 1.—Action of cow's milk, raw and heated to various temperatures, on *B. mucosus*.

plates prepared from each tube at intervals of two hours. The plates remained in the incubator at 37° C. for three days when the colonies were counted.

As soon as 6 c.c. or more of filtrate were obtained from the milk filtering through the Berkefeld, the test for agglutination was commenced. Small tubes of even caliber were sterilized and dilutions of the serum prepared so that each tube, after adding the suspension of bacteria, contained 4 c.c. The dilutions prepared were 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, and 1:1,000. Observations were taken after 2, 4, and 20 hours. It was found that dilutions of 1:2 and 1:5 contained so much milk serum, that the bacteria multiplied too rapidly and agglutination could not be recognized. In all cases, excepting with *B. coli*, there was no appreciable agglutination within the first four hours and we have therefore omitted these results from the table. No agglutination was observed in dilutions of 1:1,000, and only in one case 1:500. The suspension of bacteria employed in the agglutination tests was prepared from the same 24-hour agar culture which was inoculated into the milk for plating.

Several points of interest can be recorded by a detailed study of the table of results appended:

1. By adding the figures and calculating averages, we find that, although there is a decrease of numbers of bacteria up to two hours and a half, and even to four hours and a half in some samples of milk, this decrease is counterbalanced by an increase in the number in other samples, so that a curve, as shown in the accompanying chart, shows a slight increase. This increase is more pronounced after four hours and a half and becomes still more so after six hours and a half and eight hours and a half. The average number of bacteria in the inoculated milk, however, shows a decided decrease for at least two hours and a half, after which period there is a gradual increase. It must be remembered in this connection that by keeping the milk at 37° C. the so-called germicidal action is of shorter duration than if the milk is kept at a lower temperature.

The milk heated to 56° C. and inoculated with bacterial suspensions shows a steadily ascending curve, if the average is taken. This curve rises but slowly for the first two hours and a half, but quite rapidly after four hours and a half. The average number of bacteria in the milk heated to 75° C. and 100° C. rises more rapidly than in the milk heated to 56° C. for the first two hours and a half, and the curve ascends very decidedly after this period. The difference in the curves of the numbers of bacteria at 75° C. and 100° C. is slight and but one curve has been plotted on the chart.

2. The differences in counts of bacteria from milk shaken moderately and milk shaken vigorously are quite insignificant, the only

appreciable difference being found in the cases of *B. pyocyaneus* in raw milk and *B. prodigiosus* in milk heated to 56° C. *B. pyocyaneus* increases if shaken vigorously, but decreases if shaken moderately.

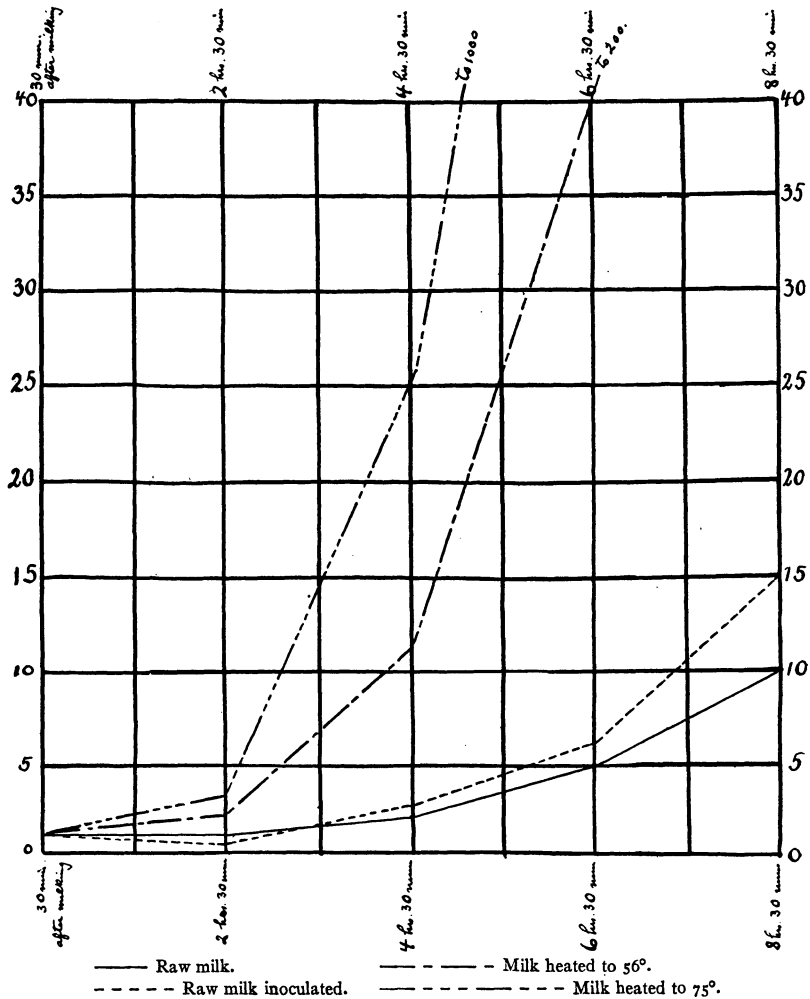


CHART 1.—Relative Growth of Bacteria in Raw Milk, Raw Milk Inoculated, and Inoculated Milk, Previously Heated to 56° and 75° C.

Sp. cholerae decreases in both, but the decrease is more pronounced if shaken moderately. These results do not exclude the possibility, that we might have found more difference if the vigorous shaking

had been done by means of some mechanical device rather than by hand. When moderately shaken the flask was rotated gently until the mixture was homogeneous; when vigorously shaken the flask was shaken 50 to 75 times.

3. If we study the decrease or increase of the various species individually we find that in raw milk *B. coli*, *B. pyocyaneus*, *B. aërogenes*, *B. mucosus*, *B. dysenteriae*, and *Staph. aureus* decrease in numbers for at least two hours and a half, but that there is a steady increase after four hours and a half. *Bacillus No. 2* from milk, *B. cloacae*, *B. cholerae-suis*, and *B. typhosus* decrease for at least two hours and a half with an increase after six hours and a half. *B. prodigiosus* and *Sp. cholerae* decrease for at least six hours and a half, and *Sarcina lutea* stands in the isolated position of showing a decrease after eight hours and a half. When heating the milk to 56° C. for 30 minutes the *Bacillus No. 2* from milk decreases for at least two hours and a half after which time there is a decided increase, and *Sarcina lutea* decreases for at least eight hours and a half. In the milk heated to 75° C. we have an increase of numbers in all species excepting *Sarcina lutea* from the beginning. This organism decreases for two hours and a half before an increase is noted. Finally *Strept. lacticus* occupies the the unique position of increasing from the start even in raw milk.

4. The point of chief interest brought out by this work is the relation of agglutination of certain bacteria by milk serum to the decrease of numbers of the same species. The results are consistent on the whole, although there are some exceptions. It must be remembered, however, that milk is rather difficult to filter through a Berkefeld filter and we have found that the time required for filtering varies greatly with different milks and different filters. In some instances we obtained enough serum after an hour and a half; in others eight hours were required to yield enough serum to work with. It is to be presumed that, as the time for filtration is prolonged, the agglutinins, which may be present in the milk, are active and are consequently consumed, so that the tube reaction becomes less marked in proportion to the time required for filtration.

Good agglutination results were obtained with *B. coli*, *B. aërogenes*, *B. mucosus*, *Staph. aureus*, *Sp. cholerae*, and *B. typhosus*. All

these organisms excepting *B. typhosus* showed a decided decrease in numbers, so that the agglutination phenomenon accounts for this decrease satisfactorily. *Bacillus* No. 2 from milk decreased markedly, although the agglutination reaction was insignificant. Unfortunately our records do not show the time consumed in obtaining a sufficient amount of serum when working with *B. typhosus* and *Bacillus* No. 2 from milk.

B. prodigiosus, *Bacillus* No. 1 from milk, and *B. pyocyaneus* decreased but moderately, and in harmony with this fact is that of the moderate agglutination of these organisms. Enough serum was obtained in two or three hours for testing these cultures. *B. cloacae* also decreased moderately. It required five hours to obtain enough serum and the agglutination was wholly negative. *B. chol.-suis* decreased considerably, and it took six hours to obtain enough serum to work with. The agglutination was quite moderate. *Sarcina lutea* decreased considerably, although the agglutination was but fair. In this case the filtering process lasted for five hours.

Milk serum seems to possess an exceptionally marked agglutinative influence on *Sp. cholerae*. This organism agglutinated partially in a dilution of 1:500 and completely in a dilution 1:200. The serum was obtained in an hour and a half. The decrease of this organism in milk is more decided than that of any other species used in the experiments. This observation seems to agree with a statement made by Hesse¹ who found that milk is a poor medium for cholera spirilla and that they died before sufficient acid was formed in the milk to affect them.

We find an exceptional phenomenon with *Strept. lacticus*, which increases markedly from the outset even in raw milk and at the same time is agglutinated considerably. It is difficult to form a theory to explain this. *Strept. lacticus* is usually found in but small numbers in fresh milk and it is possible that it multiplies with such rapidity that the agglutination of the milk is not sufficient to effect a visible decrease. This theory is supported somewhat by the fact that the increase is considerably slower in raw milk than in milk heated to 56° C., and the difference is still more pronounced in milk heated to 75° or 100° C. It has also been shown² that *Strept. lacticus* increases

¹ Loc. cit.

² Heinemann, *Jour. of Infect. Dis.*, 1906, 3, p. 192.

TABLE I.
EXPERIMENTS WITH THE SO-CALLED GERMICIDAL ACTION OF FRESH COW'S MILK.

SERIES	ORGANISM INOCULATED	TIME OF PLATING AFTER MILKING	NUMBER OF BACTERIA IN CC RAW MILK		NUMBER OF BACTERIA IN CC MILK AFTER INOCULATION						
			Shaken moderately	Shaken vigorously	Raw Milk Kept. at 37° C.		Milk Heated to 56° C. for 30 Min.		Milk Heated to 75° C. for 30 Min.		Milk Heated to 100° C. for 30 Min.
					Shaken moderately	Shaken vigorously	Shaken moderately	Shaken vigorously	Shaken moderately	Shaken vigorously	Shaken moderately
1	{ Bacillus isolated from milk	30 min.	500	600	60,000	70,000	50,000	70,000	60,000	60,000	60,000
		2 hrs. 30 min.	400	500	30,000	50,000	320,000	340,000	850,000	920,000	860,000
		4 hrs. 30 min.	300	500	40,000	60,000	520,000	860,000	1,320,000	1,350,000	2,640,000
		6 hrs. 30 min.	600	700	120,000	130,000	780,000	990,000	2,100,000	3,000,000	12,600,000
		8 hrs. 30 min.	1,000	1,100	3,550,000	5,400,000	1,340,000	1,450,000			
2	{ Bacillus isolated from milk	30 min.	0	400	20,000	30,000	40,000	20,000	20,000	40,000	20,000
		2 hrs. 30 min.	0	0	0	0	10,000	10,000	40,000	50,000	40,000
		4 hrs. 30 min.	0	0	10,000	20,000	40,000	90,000	120,000	130,000	120,000
		6 hrs. 30 min.	0	0	30,000	120,000	500,000	700,000			
		8 hrs. 30 min.	100	200	650,000	790,000					
3	B. coli	30 min.	1,100	1,100	1,350,000	1,800,000	1,430,000	950,000	1,050,000	540,000	530,000
		2 hrs. 30 min.	200	100	380,000	780,000	5,500,000	4,900,000	6,800,000	8,200,000	7,600,000
		4 hrs. 30 min.	0	800	9,800,000	1,200,000					
		6 hrs. 30 min.	300	6,200	15,200,000	18,800,000					
		8 hrs. 30 min.	4,500	6,700							
4	{ B. cloacae from a bird	30 min.	1,000	2,600	800,000	940,000	740,000	800,000	560,000	1,200,000	760,000
		2 hrs. 30 min.	1,000	2,600	620,000	720,000	1,800,000	1,540,000	6,100,000	6,650,000	5,100,000
		4 hrs. 30 min.	400	100	410,000	450,000	11,100,000	11,000,000			
		6 hrs. 30 min.	200	200	3,450,000	3,400,000	17,950,000	18,000,000			
		8 hrs. 30 min.	1,000	500	10,100,000	10,600,000					
5	Sarcina lutea	30 min.	200	200	320,000	340,000	210,000	400,000	220,000	230,000	320,000
		2 hrs. 30 min.	200	200	80,000	240,000	120,000	200,000	370,000	370,000	460,000
		4 hrs. 30 min.	200	200	40,000	40,000	110,000	120,000	450,000	460,000	280,000
		6 hrs. 30 min.	100	100	40,000	70,000	110,000	190,000	480,000	470,000	250,000
		8 hrs. 30 min.	100	100	1,000	100,000	38,000	380,000	500,000	530,000	300,000
6	B. prodigiosus	30 min.	500	800	760,000	680,000	830,000	680,000	850,000	800,000	1,050,000
		2 hrs. 30 min.	1,300	1,300	320,000	360,000	800,000	1,030,000	1,120,000	1,090,000	1,080,000
		4 hrs. 30 min.	1,800	1,700	460,000	320,000	1,800,000	1,370,000	2,210,000	1,750,000	1,700,000
		6 hrs. 30 min.	2,300	3,300	360,000	420,000	6,100,000	6,900,000			
		8 hrs. 30 min.	3,200	4,300	400,000	520,000					
7	B. pyocyaneus	30 min.	600	800	1,640,000	880,000	1,100,000	1,210,000	1,200,000	1,090,000	1,090,000
		2 hrs. 30 min.	200	700	1,040,000	1,090,000	1,470,000	1,300,000	1,560,000	1,200,000	1,200,000
		4 hrs. 30 min.	100	200	2,200,000	3,100,000	4,150,000	4,200,000	5,200,000	4,500,000	4,500,000
		6 hrs. 30 min.	200	200	6,950,000	6,800,000					
		8 hrs. 30 min.	200	400							
8	B. aerogenes	30 min.	400	300	420,000	580,000	560,000	430,000	440,000	580,000	570,000
		2 hrs. 30 min.	300	300	260,000	280,000	610,000	840,000	1,500,000	2,150,000	1,500,000
		4 hrs. 30 min.	200	200	300,000	450,000	3,430,000	3,730,000	5,120,000	5,200,000	8,500,000
		6 hrs. 30 min.	100	100	620,000	1,040,000	8,200,000	10,100,000			
		8 hrs. 30 min.	100	400	1,900,000	3,100,000					
9	{ B. cholerae-suis	30 min.	1,400	2,400	210,000	180,000	320,000	290,000	230,000	240,000	102,000
		2 hrs. 30 min.	500	200	42,000	160,000	490,000	500,000	600,000	620,000	601,000
		4 hrs. 30 min.	400	100	50,000	70,000	1,050,000	1,070,000	1,610,000	1,630,000	1,610,000
		6 hrs. 30 min.	300	1,500	560,000	860,000					
		8 hrs. 30 min.	900	2,100	3,600,000	4,200,000					
10	B. mucosus	30 min.	300	300	170,000	430,000	210,000	360,000	180,000	310,000	210,000
		2 hrs. 30 min.	0	200	60,000	80,000	380,000	460,000	1,110,000	1,390,000	1,430,000
		4 hrs. 30 min.	800	1,200	390,000	400,000	1,760,000	2,200,000			
		6 hrs. 30 min.	1,000	1,800	14,000,000	14,500,000					
		8 hrs. 30 min.	1,100	1,900							
11	B. typhosus	30 min.	500	800	290,000	320,000	220,000	340,000	320,000	250,000	320,000
		2 hrs. 30 min.	1,400	2,200	260,000	250,000	250,000	480,000	330,000	350,000	380,000
		4 hrs. 30 min.	3,400	4,100	150,000	320,000	1,030,000	1,600,000	1,580,000	1,580,000	1,490,000
		6 hrs. 30 min.	5,600	5,800	210,000	220,000	5,140,000	5,500,000	6,760,000	6,010,000	8,600,000
		8 hrs. 30 min.	6,300	7,900	1,400,000	2,220,000	14,500,000	15,800,000			
12	B. dysenteriae	30 min.	2,200	1,600	610,000	240,000	470,000	230,000	520,000	560,000	420,000
		2 hrs. 30 min.	4,500	2,900	30,000	80,000	480,000	350,000	750,000	820,000	580,000
		4 hrs. 30 min.	21,000	15,000	90,000	130,000	5,700,000	4,500,000	7,700,000	8,100,000	8,200,000
		6 hrs. 30 min.	28,000	31,000	120,000	200,000					
		8 hrs. 30 min.	36,000	46,000	620,000	640,000					
13	Staph. aureus	30 min.	200	500	310,000	320,000	370,000	80,000	94,000	140,000	420,000
		2 hrs. 30 min.	200	300	140,000	160,000	520,000	160,000	120,000	360,000	760,000
		4 hrs. 30 min.	2,200	3,700	510,000	920,000	630,000	340,000	320,000	870,000	1,750,000
		6 hrs. 30 min.	10,800	13,500	2,300,000	3,400,000	4,300,000	4,300,000	2,400,000	4,300,000	3,740,000
		8 hrs. 30 min.	45,000	56,500	4,100,000	5,300,000	6,200,000	5,500,000	4,700,000	6,500,000	6,400,000
14	Strep. lacticus	30 min.	700	400	21,000	24,000	219,000	12,000	15,000	16,000	21,000
		2 hrs. 30 min.	500	100	74,000	31,000	43,000	54,000	120,000	130,000	64,000
		4 hrs. 30 min.	100	100	250,000	240,000	84,000	1,100,000	1,780,000	1,810,000	1,980,000
		6 hrs. 30 min.	200	300	296,000	350,000	510,000	6,600,000			
		8 hrs. 30 min.	2,100	3,400	1,700,000	1,900,000					
15	Spir. cholerae	30 min.	200	300	190,000	230,000	64,000	140,000	180,000	110,000	52,000
		2 hrs. 30 min.	100	100	40,000	61,000	41,000	160,000	40,000	82,000	120,000
		4 hrs. 30 min.	200	300	11,000	0	130,000	240,000	260,000	310,000	340,000
		6 hrs. 30 min.	300	2,100	0	0	140,000	120,000	280,000	360,000	970,000
		8 hrs. 30 min.	3,300	7,000	12,000	32,000	50,000	50,000	310,000	460,000	1,400,000

TABLE I.
EXPERIMENTS WITH THE SO-CALLED GERMICIDAL ACTION OF FRESH COW'S MILK.

BACTERIA MILK	NUMBER OF BACTERIA IN CC MILK AFTER INOCULATION								AGGLUTINATION IN MILK SERUM AFTER 20 HOURS					
	Raw Milk Kept. at 37° C.		Milk Heated to 56° C. for 30 Min.		Milk Heated to 75° C. for 30 Min.		Milk Heated to 100° C. for 30 Min.		DILUTION 1:					
	Shaken moderately	Shaken vigorously	Shaken moderately	Shaken vigorously	Shaken moderately	Shaken vigorously	Shaken moderately	Shaken vigorously	5	10	20	50	100	200
600	60,000	70,000	50,000	70,000	60,000	60,000	60,000	90,000	+	+	+			
500	30,000	50,000	320,000	340,000	850,000	920,000	800,000	900,000						
500	40,000	60,000	520,000	860,000	1,320,000	1,350,000	2,040,000	3,850,000						
700	120,000	130,000	780,000	990,000	2,100,000	3,000,000	12,000,000	15,000,000						
1,100	3,550,000	5,400,000	1,340,000	1,450,000										
400	20,000	30,000	40,000	20,000	20,000	40,000	20,000	10,000	+	+				
0	0	0	10,000	10,000	40,000	50,000	40,000	50,000						
0	10,000	20,000	40,000	90,000	120,000	130,000	120,000	150,000						
0	30,000	120,000	500,000	760,000										
200	650,000	790,000												
1,100	1,350,000	1,800,000	1,430,000	950,000	1,050,000	540,000	530,000	1,100,000	+	+	+	+	+	+
100	380,000	780,000	5,500,000	4,900,000	6,800,000	8,200,000	7,000,000	9,700,000						
800	9,800,000	1,200,000												
6,200	15,200,000	18,800,000												
6,700														
2,600	800,000	940,000	740,000	800,000	560,000	1,200,000	760,000	740,000						
2,600	620,000	720,000	1,800,000	1,540,000	6,100,000	6,050,000	5,100,000	6,500,000						
100	410,000	450,000	11,100,000	11,000,000										
200	3,450,000	3,400,000	17,950,000	18,000,000										
500	10,100,000	10,600,000												
200	320,000	340,000	210,000	400,000	220,000	230,000	320,000	330,000						
200	80,000	240,000	120,000	200,000	370,000	370,000	400,000	540,000	+	+	+			
200	40,000	40,000	110,000	120,000	450,000	400,000	280,000	420,000						
100	40,000	70,000	110,000	190,000	480,000	470,000	250,000	280,000						
100	1,000	100,000	38,000	380,000	500,000	530,000	300,000	420,000						
800	760,000	680,000	830,000	680,000	850,000	800,000	1,050,000	830,000						
1,300	320,000	360,000	800,000	1,030,000	1,120,000	1,090,000	1,080,000	1,060,000	+	+	+			
1,700	460,000	320,000	1,180,000	1,370,000	2,210,000	1,750,000	1,700,000	2,140,000						
3,300	360,000	420,000	6,100,000	6,900,000										
4,300	400,000	520,000												
800	1,640,000	880,000	1,100,000	1,210,000	1,200,000	1,090,000	1,090,000	1,290,000	+	+	+			
700	1,040,000	1,090,000	1,470,000	1,300,000	1,560,000	1,200,000	1,200,000	1,400,000						
200	2,200,000	3,100,000	4,150,000	4,200,000	5,200,000	4,500,000	4,500,000	5,100,000						
200	6,950,000	6,800,000												
400														
300	420,000	580,000	560,000	430,000	440,000	580,000	570,000	290,000	+	+	+	+	+	+
300	260,000	280,000	610,000	840,000	1,500,000	2,150,000	1,500,000	2,200,000						
200	300,000	450,000	3,430,000	3,730,000	5,120,000	5,200,000	8,500,000	9,500,000						
100	620,000	1,040,000	8,200,000	10,100,000										
400	1,900,000	3,100,000												
2,400	210,000	180,000	320,000	290,000	230,000	240,000	102,000	160,000	+	+	+			
200	42,000	160,000	490,000	500,000	600,000	620,000	601,000	610,000						
100	59,000	70,000	1,050,000	1,670,000	1,610,000	1,630,000	1,010,000	1,940,000						
1,500	560,000	860,000												
2,100	3,600,000	4,200,000												
300	170,000	430,000	210,000	360,000	180,000	310,000	210,000	320,000	+	+	+	+	+	+
200	60,000	80,000	380,000	460,000	1,110,000	1,390,000	1,430,000	1,720,000						
1,200	390,000	400,000	1,760,000	2,200,000										
1,800	14,000,000	14,500,000												
1,900														
800	200,000	320,000	220,000	340,000	320,000	250,000	320,000	310,000	+	+	+	+	+	+
2,200	260,000	250,000	250,000	480,000	330,000	350,000	380,000	430,000						
4,100	150,000	320,000	1,030,000	1,600,000	1,580,000	1,580,000	1,400,000	1,700,000						
5,800	210,000	220,000	5,140,000	5,500,000	6,760,000	6,010,000	8,600,000	8,400,000						
7,900	1,400,000	2,220,000	14,500,000	15,800,000										
1,600	610,000	240,000	470,000	350,000	750,000	820,000	580,000	650,000	+	+	+			
2,900	30,000	80,000	5700,000	4,500,000	7,700,000	8,100,000	8,200,000	8,500,000						
5,000	90,000	130,000												
11,000	120,000	200,000												
16,000	620,000	640,000												
500	310,000	340,000	370,000	80,000	94,000	140,000	420,000	240,000	+	+	+	+	+	+
300	140,000	160,000	520,000	160,000	120,000	360,000	760,000	820,000						
3,700	510,000	920,000	630,000	340,000	320,000	870,000	1,750,000	1,700,000						
3,350	2,300,000	3,400,000	4,300,000	4,300,000	2,400,000	4,300,000	3,740,000	4,850,000						
16,500	4,100,000	5,300,000	6,200,000	5,500,000	4,700,000	6,500,000	6,400,000	7,500,000						
400	21,000	24,000	210,000	12,000	15,000	16,000	21,000	13,000	+	+	+	+	+	+
100	74,000	31,000	43,000	54,000	120,000	130,000	64,000	120,000						
100	250,000	240,000	84,000	1,100,000	1,780,000	1,810,000	1,980,000	2,100,000						
300	296,000	359,000	510,000	6,600,000										
3,400	1,700,000	1,900,000												
300	100,000	230,000	64,000	140,000	180,000	110,000	52,000	240,000	+	+	+	+	+	+
100	40,000	61,000	41,000	160,000	40,000	82,000	120,000	260,000						
300	11,000	0	130,000	240,000	260,000	310,000	340,000	360,000						
2,100	0	0	140,000	120,000	280,000	360,000	970,000	1,100,000						
7,000	12,000	32,000	50,000	50,000	310,000	460,000	1,460,000	2,720,000						

in milk to such an extent as to suppress practically all other organisms.

SUMMARY AND CONCLUSIONS.

1. The decrease of bacteria in fresh cow's milk is more decided if fairly large numbers are inoculated than if small numbers only are present.
2. The relative increase of bacteria in milk is more pronounced in milk heated to 75° C. or 100° C. than in raw milk or in milk heated to 56° C.
3. The difference in the relative decrease in numbers of bacteria in milk moderately shaken and vigorously shaken is not marked if this shaking is done by hand. Some difference was observed, however, and this difference might be more pronounced if the milk were shaken more violently.
4. Some species occurring naturally in milk decrease considerably in numbers during the first four or five hours, some decrease slightly, some hold their own or even increase.
5. Milk inoculated with pure cultures of bacteria seems to restrain to a marked degree the multiplication of these bacteria for several hours at 37° C. and for a somewhat longer period at room temperature, excepting in the case of *Strept. lacticus*, which increases from the beginning, although it may be inhibited to some extent.
6. Heating milk to 56° C. for 30 minutes does not entirely destroy the power to restrain the multiplication of bacteria; this power is weakened however, and at 75° C. is destroyed completely. This fact together with the fact that milk serum agglutinates some species of bacteria *in vitro* to a marked degree seems to favor the assumption that agglutinins are in part responsible for the apparent decrease of bacteria in fresh milk, since bactericidal substances are destroyed by heating to 56° C. for 30 minutes.
7. The agglutination of certain bacteria in milk serum seems to bear some relation to the apparent decrease in numbers of bacteria observed in fresh milk, but this is probably not the only factor causing such reduction.